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Serum levels of ghrelin, adipokines, and tumor necrosis factor-α (TNF-α) in patients with juvenile idiopathic arthritis in Assuit University Hospitals: Relation to nutritional status and disease activity

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KEYWORDS

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Ghrelin;
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Abstract *Background:* In juvenile idiopathic arthritis (JIA) several proinflammatory cytokines are secreted leading to anorexia and weight loss.
Objectives: We aimed to measure serum levels of ghrelin, adipocytokines and tumor necrosis factor alpha (TNF-α) in JIA patients. Also, to correlate them with disease activity and patients' body mass index (BMI).
Patients and methods: Sixty-five patients with JIA (40 girls and 25 boys; means ± SD of age were 12.1 ± 3.5 years) and 54 matched healthy controls were evaluated by ELISA to measure levels of ghrelin, leptin, adiponectin and TNF-α and correlate these levels with Juvenile Arthritis Disease Activity Score (JADAS) and patients' BMI.
Results: Thirty-four patients (52.3%) had active disease while 31 (47.7%) had inactive disease. JIA patients had significantly lower level of ghrelin and leptin and significantly higher level of TNF-α than the control group ($P \leqslant 0.001$, $P \leqslant 0.05$, $P \leqslant 0.001$, respectively). Those with active disease had significantly lower level of ghrelin and significantly higher TNF-α level than those with inactive disease ($P \leqslant 0.001$, $P \leqslant 0.01$, respectively). Significant positive correlations were found between ghrelin and leptin levels and patients' BMI ($P \leqslant 0.05$, $P \leqslant 0.01$, respectively).

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Conclusion: Ghrelin and leptin could be considered to be responsible for decreased BMI in patients with JIA, so they might be potential targets of new therapeutic strategies for correction of anorexia and associated weight loss in those patients. Low ghrelin and high TNF-α could be used as useful tools in monitoring disease activity in JIA.

Introduction

Juvenile idiopathic arthritis (JIA) is a term that collectively refers to a group of chronic arthropathies, which together constitute the most common rheumatic condition in children. It is also an inflammatory joint disease characterized by chronic synovitis and associated with extra-articular manifestation such as, fever, anorexia, weight loss, anemia, lymphadenopathy, pericarditis, and uveitis.[1]

Ghrelin, a recently discovered hormone, is produced primarily by cells in the stomach but is also expressed in white adipose tissue.[2] It has been shown to affect a number of different systems including growth hormone release, feeding, gastric acid secretion, gastric motility, and cell proliferation. Ghrelin and its receptor have been identified in T cells. It is a potent anti-inflammatory mediator in lymphocytes, monocytes and dendritic cells. It inhibits oxidative stress, cellular apoptosis, cell adhesion and proinflammatory cytokine expression and promotes IL-10 expression and cell migration.[3]

Adipokines (adipose tissue cytokines), including leptin and adiponectin are emodeling hormones produced mainly by adipose tissue. Leptin regulates food intake and energy balance. It is now regarded as a pivotal factor in the interplay between neuroendocrine function and the immune system.[4,5] Proinflammatory cytokines increase circulating leptin, which in turn triggers cytokine release in monocytes/macrophages and stimulates T cell-mediated immunity during acute inflammation.[6,7] However, leptin may also limit the inflammatory response.[8,9]

Adiponectin, is postulated to be associated in the modulation of inflammatory responses as it attenuates the inflammatory response mediated by TNF-α, inhibits macrophage phagocytic activity and TNF-α production.[10]

Cytokines are directly implicated in many of the immune processes that are associated with the pathogenesis of JIA. The most important proinflammatory cytokines produced by phagocytes are tumor necrosis factor alpha (TNF-α), IL-1 and IL-6; the key cytokines that drive inflammation in JIA.[11]

TNF-α is a proinflammatory cytokine produced as a membrane-bound 26 kDa molecule from which the soluble 17 kDa active TNF-α molecule is released by the TNF-α converting enzyme.[12] The circulating TNF-α levels are highly variable.[13] Also, TNF-α is involved in several biologic processes such as tissue emodeling, epithelial cell barrier permeability, macrophage activation, recruitment of inflammatory cells, effectiveness of the local and systemic inflammation and amplification of other proinflammatory cytokine actions.[14–16]

Anorexia and weight loss are consistent clinical manifestations during acute and chronic inflammatory processes, which are accompanied by decreased quality of life and survival. These changes are generally attributed to the increased levels of circulating cytokines such as TNF-α, interleukins 1 (IL1) and 6 (IL6).[1]

As JIA is an inflammatory disease that is frequently accompanied by anorexia and weight loss to our knowledge there are a few studies regarding serum level of ghrelin and adipokines, which is related to appetite and inflammation. So, the aim of our study is to investigate circulating serum level of ghrelin and adipose tissue cytokines (leptin, adiponectin) and a proinflammatory cytokine (TNF-α) in JIA patients. Also to correlate these levels with Juvenile Arthritis Disease Activity Score (JADAS) and patients' BMI.

Patients and methods

Sixty-five patients with JIA (40 girls and 25 boys) aged from 4 to 16 years and have disease duration ranging from 2 to 144 months were enrolled in this study. They were selected from Rheumatology & Rehabilitation and Pediatric Departments in Assuit University Hospitals. Classification of JIA patients was done according to The International League of Associations for Rheumatology (ILAR) criteria.[17]

Treatment of JIA patients with disease-modifying antirheumatic drugs (DMARDS) and suitable dosage of nonsteroidal anti-inflammatory drugs (NSAIDS) during the study was allowed. Fifty-four healthy children matched with the patients according to age, sex were recruited as control group.

Exclusion criteria Patients having diabetes mellitus, endocrinal disease (Cushing syndrome, thyroid diseases), patients taking corticosteroid during the last 6 months and those having any malignancy were excluded.

Informed consent was obtained from all children's parents and the study approved by ethics committee of faculty of medicine, Assiut University.

All patients and controls were subjected to the following

A. Detailed history taking.
B. Rheumatologic and systemic examination.
C. BMI was calculated as weight [in kilograms] divided by squared height [in meters] (kg/m^2). BMI below 5th percentile reflects underweight, BMI between 5th and 84th percentile reflects normal weight, BMI between 85th percentile and 94th percentile reflects at risk for overweight and BMI above 95th percentile reflects overweight.[18]
D. Assessment of JIA activity (in patients): by Juvenile Arthritis Disease Activity Score (JADAS).[19,20]
E. Laboratory assessment: complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), measurement of serum ghrelin, leptin, adiponectin and TNF-α.

6 ml of venous blood was withdrawn from each individual participating in the study and was divided as follows: 3 ml in vacutainer tubes with EDTA anticoagulant which were centrifuged immediately at 3000 rpm for 10 min for quantitative measurement of human unacylated ghrelin in plasma (Biovender RD, France). The other 3 ml of blood was transferred to a wassermann tube from which the separated serum was divided into aliquots and stored at −80 °C (for not more than 2 months) for further estimation of active human leptin by ELISA (Diagnostic System Laboratories, Inc.), human adiponectin by ELISA (Orgenium, Finland) and TNF-α done by ELISA (Biosource, Europe SA) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS software (version 16). The values are expressed as mean ± SD, median, or percentage as appropriate. For comparison between groups, Chi square test was used for categorical variables, unpaired *T*-test and Mann–Whitney *U*-test was used for numerical variables. Spearman's rho correlation was used to assess the relationship between laboratory parameters. Differences were considered significant at $P < 0.05$.

Table 1 Characteristics of the studied patients with juvenile idiopathic arthritis.

Variable	Patients ($n = 65$)
	No. (%)
Age (years)	
Mean ± SD (range)	12.1 ± 3.5 (4–16)
Sex	
Males	25 (38.5)
Females	40 (61.5)
Pattern of JIA	
Systemic onset	16 (24.6)
Polyarticular	16 (24.6)
Oligoarticular	14 (21.5)
ERA*	15 (23.1)
Psoriatic arthritis	4 (6.2)
Disease duration (months)	
Mean ± SD (range)	49.6 ± 34.5 (2–144)
Activity	
Active	34 (52.3)
Not active	31 (47.7)

* ERA, enthesitis related arthritis.

Results

Table 1 shows the demographic characteristics of JIA patients. The mean ± SD of age were 12.1 ± 3.5 years, females predominate (61.5%), the type of JIA onset were 16 patients (24.6%) with systemic onset, 16 (24.6%) polyarticular, 14 (21.5%) oligoarticular, 15 (23.1%) enthesitis related arthritis (ERA) and 4 (6.2%) psoriatic arthritis. Disease duration mean ± SD was 49.6 ± 34.5. Regarding activity of the disease 34 patients (52.3%) had active disease while 31 (47.7%) had inactive disease.

Table 2 shows serum levels of ghrelin, leptin, adiponectin, and TNF-α in patients (active and inactive) and controls. It reveals that JIA patients had significantly lower serum levels of ghrelin and leptin and significantly higher TNF-α level than the control group ($P \leqslant 0.001$, $P \leqslant 0.05$, $P \leqslant 0.001$, respectively), while there was no significant difference regarding serum level of adiponectin in JIA patients and controls. In addition, Table 2 also shows significantly lower ghrelin level and significantly higher TNF-α level in those with active disease than in those with inactive disease ($P \leqslant 0.001$, $P \leqslant 0.01$, respectively), while no significant difference was detected between them regarding serum levels of leptin and adiponectin.

Fig. 1 clarifies the body mass index of patients and controls. It reveals that by calculating BMI for patients and controls, 47.1%, 64.5% and 75.9% of those patients with active, inactive disease and controls respectively have normal weight (BMI 5th–84th percentile) while 52.9%, 35.5% and 9.3% of them respectively have underweight (BMI less than 5th percentile). At risk for overweight (BMI 85th–94th percentile) and overweight (BMI more than 59th percentile) were observed only in 14.8% of controls and not in patients.

Table 3 summarizes the correlations between ghrelin, leptin, adiponectin and TNF-α levels with BMI. It reveals significant positive correlations between ghrelin and leptin with BMI ($P \leqslant 0.05$, $P \leqslant 0.01$, respectively) while no significant correlation was found between adiponectin and TNF-α with BMI.

Discussion

Ghrelin is a powerful, endogenous orexigenic peptide. In addition, ghrelin has anti-inflammatory effects, and it has been reported that ghrelin down-regulates pro-inflammatory cytokines, including interleukin (IL)-1 beta and TNF-α.[21]

Li et al. demonstrated that ghrelin attenuated TNF alpha induced nuclear translocation NF-κB, indicating that blockade for activation of the transcription factor NF-κB could be a potential mechanism whereby ghrelin modulates inflammatory responses.[22]

Our study demonstrated significantly decreased serum level of circulating ghrelin in patients with JIA compared to controls, in addition those with active disease had significantly lower level of ghrelin than those with inactive disease, and serum ghrelin was found to be correlated positively with BMI in patients with JIA. In agreement with our results, Karagiozoglou et al. reported decreased ghrelin level in patients with juvenile idiopathic arthritis, and also showed a significant relationship between ghrelin and disease activity, but they did not find a significant relationship between ghrelin and nutritional status,[23] while El-Eshmawy et al. reported negative correlation between serum ghrelin and BMI of Egyptian adolescents.[24] Our data indicate that ghrelin may play an important role in ameliorating pathologic inflammatory states.

Contradictory to our results, Sanem Eren et al. demonstrated no significant difference between JIA patients and controls as regards serum level of ghrelin[25] and Koca et al.[21] did not find significant relation between serum level of ghrelin and disease activity in patients with JIA.

Other researchers have found significant decreased ghrelin levels in patients with rheumatoid arthritis and in a rat

Table 2 Serum levels of ghrelin, leptin, adiponectin, and TNF-α in patients (active and inactive) and controls.

Studied parameter Mean ± SD Range	Patients			Controls (No. = 54)	*P* value	
	Active JIA (No. = 34)	Inactive JIA (No. = 31)	Total patients (No. = 65)		Patients vs controls	Active JIA vs inactive JIA
Ghrelin (pg/ml)	30.2 ± 4.5 23.0–42.0	45.3 ± 8.3*** 29.0–65.0	37.4 ± 10.1 23.0–65.0	112.9 ± 15.7*** 75.0–137.0	< 0.001	< 0.001
Leptin (ng/ml)	19.2 ± 6.2 9.2–30.0	18.8 ± 4.9 10.0–29.0	19.0 ± 5.6 9.2–30.0	22.3 ± 8.1* 7.3–42.5	< 0.05	NS
Adiponectin (μg/ml)	64.2 ± 20.1 27.7–89.0	64.5 ± 19.7 24.0–88.6	64.3 ± 19.8 24.0–89.0	67.2 ± 17.9 24.0–89.4	NS	NS
TNF-α (pg/ml)	48.5 ± 6.7 35.0–61.0	43.6 ± 4.9** 36.7–52.2	46.2 ± 6.4 35.0–61.0	25.9 ± 2.8*** 20.5–30.3	< 0.001	< 0.01

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS = Non significant.

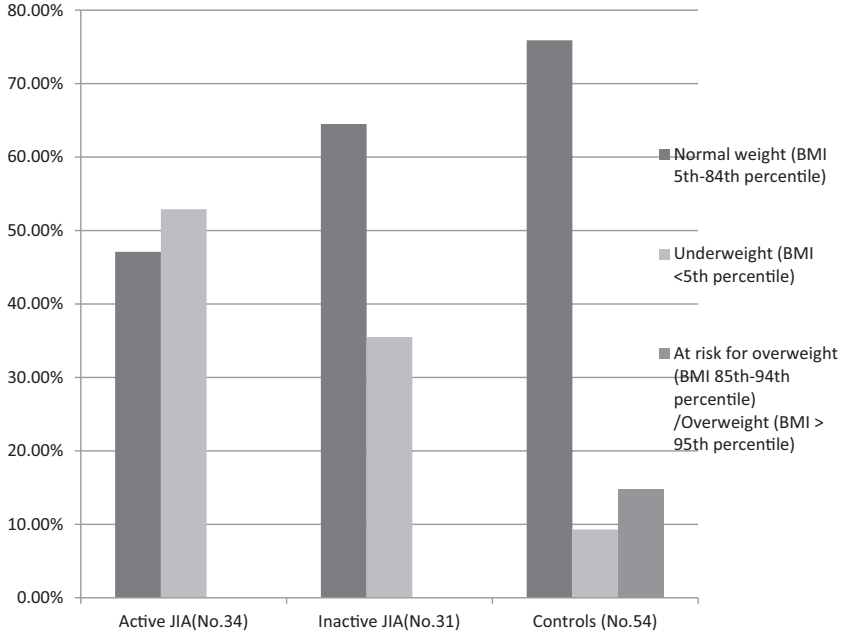

Figure 1 Body mass index of juvenile idiopathic arthritis patients (active and inactive) and controls.

Table 3 Correlations between ghrelin, leptin and TNF-α, adiponectin with body mass index (BMI) in studied patients.

	BMI	
	r-Value	*P*-value
Ghrelin (pg/ml)	0.273	< 0.05
Leptin (ng/ml)	0.545	< 0.001
Adiponectin (μg/ml)	0.088	0.487
TNF-α (pg/ml)	−0.36	0.776

adjuvant-induced arthritis model and they mentioned that low ghrelin concentrations could reflect the presence of an inhibitory anorexigenic signal/s.[21,26]

Leptin is a novel proinflammatory adipocyte derived factor that operates in the cytokine network by linking immune and inflammatory processes to the neuroendocrine. A positive correlation between BMI and circulating leptin concentration has been previously reported in Egyptian patients with growth delay[24] or with JIA.[27,28]

Our study demonstrated decreased serum level of leptin in patients with JIA than controls and no relation was detected with disease activity but a significant positive correlation was detected between serum leptin level in JIA patients and their BMI. In agreement with our results, other studies demonstrated that plasma leptin in JIA patients was significantly lower than in controls.[1,25] Also previous researchers mentioned that circulating leptin concentration was not related to the activity of the disease but significantly related to BMI of JIA patients either white children[1] or Egyptian adults.[28]

The results of the present study came also in accordance with Popa et al.[29], Lago et al.[30] and Gunaydin et al.[31] who concluded that serum leptin in RA patients reflects BMI but not joint inflammation. These findings of low leptin in patients which is not associated with increased weight could be explained by the suggestion that hypothalamus had been made insensitive to the low leptin level by the effect of

proinflammatory cytokines which may mime the hypothalamic effect of excessive negative feedback signaling from leptin, leading to the prevention of the normal compensatory mechanisms in the face of both decreased food intake and body weight.[1] On the contrary, Agata et al.[32] reported that leptin concentration in sera of JIA children was higher than in healthy children, also mentioned that in JIA children with high disease activity leptin concentration was higher than in children with low disease activity, but without statistical significance. However, they found a positive correlation between serum leptin concentration and BMI of their patients and concluded that high leptin concentration in JIA children and its correlation with BMI could indicate leptin's role in body mass regulation in the course of the chronic inflammatory process. Some researchers also reported higher level of leptin in adult Egyptian patients with JIA than in controls.[33,34] Abdalla et al. reported a positive correlation between leptin level and BMI of Egyptian adults with JIA but no correlation was found between leptin and disease activity.[34] Winiarska et al. reported no significant difference in serum level of leptin between JIA patients and controls.[35] From the results of these conflicting reports, leptin may influence JIA in opposing ways: the expression of cytokines or limiting the inflammatory responses, leptin may be a marker of nutritional status in JIA patients.

Our results demonstrated no significant difference in serum level of adiponectin in patients with JIA and healthy controls, also no significant difference in serum level of adiponectin in those with active disease and inactive disease. No correlation was found between serum level of adiponectin in patients with JIA and their BMI. Our results were in agreement with some researchers who reported normal adiponectin level in JIA patients[35–37], while others found that level of adiponectin in JIA patients was lower than in controls and related this to the inhibition of adiponectin production per unit of fat mass in the existence of the disease.[25] Similar to our results, Patjas et al. found no significant relation between serum adiponectin level in JIA patients and disease activity. Stepniak et al. found normal adiponectin level in JIA patients and did not find a relation between their BMI and adiponectin level.[38] Contradictory to our results, Alkady et al. reported that serum adiponectin was higher in 70 Egyptian adult patients with rheumatoid arthritis than controls and was higher in 39 patients with active disease and reported also that adiponectin was positively correlated with disease activity.[39]

Fantuzzi et al. reported that proinflammatory cytokines including TNF and IL-6 inhibit the production of adiponectin. Also reported that adiponectin may have a role in modulating the inflammatory response by inhibiting the expression of adhesion molecules on endothelial cells, suppressing macrophage function and inhibiting NF-κB signaling.[40]

In this study TNF-α in JIA patients was significantly higher than in the control group, also it was significantly higher in those patients with active disease than in those with inactive disease. No significant correlation was found between TNF-α and BMI of JIA patients. These results were in agreement with previous researchers who concluded that in Egyptian JIA patients TNF-α is significantly higher than in controls.[41,42] Also researchers reported higher levels in those with active disease.[42,43] TNF-α is involved in the pathogenesis of JIA.[44] Our results were contradictory to Spirchez et al. as they found no correlation with disease activity in JIA patients.[45]

Limitations of the study

The following points could be considered as limitations of the study:

- Not taking into consideration the disease activity score values.
- Not taking into consideration the JIA subtypes.
- Not presenting the results according to the gender in the JIA patients.

Conclusion and recommendations

Ghrelin and leptin could be considered to be partially responsible for weight loss in JIA, low ghrelin and high TNF-α could be considered useful tools in monitoring disease activity in JIA. We need further studies on larger scales as regards studying ghrelin and leptin as potential targets of new therapeutic strategies for correction of anorexia and associated weight loss in those patients with JIA.

Conflict of interest

None declared.

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